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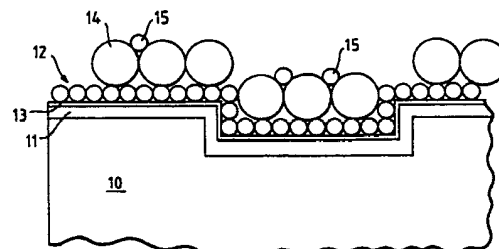
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54 Assay technique.

57 An assay technique is disclosed for the qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample. The technique comprises (a) coating at least a predetermined part of a pre-formed surface on a substrate with a thin film of a material capable of binding the species to be assayed, the pre-formed surface being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) containing the coated surface with the sample; and (c) observing the optical properties of said pre-formed surface in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material. The optical properties of the pre-formed surface may be observed before and after step (b) in order to determine any change in optical properties, or they may be monitored during step (b). The pre-formed surface is preferably a grating.

An article for use in the above technique is also disclosed, and comprises a substrate carrying said pre-formed surface which in turn is coated with the receptive material for the species to be assayed.



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1 "ASSAY TECHNIQUE"

 This invention relates to an assay technique for qualitative and/or quantitative detection of chemical, biochemical or biological species in a sample.

5 The technique is based upon the affinity of the species which is to be assayed for a receptive material, for example a ligand or a specific binding partner, which receptive material is coated onto a particular type of surface.

10 More particularly, according to one aspect of the present invention, there is provided an assay technique for qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample, which comprises (a) coating at least a predetermined part
15 of a pre-formed surface on a substrate with a thin film of a material capable of binding the species to be assayed, the pre-formed surface being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) contacting the coated surface
20 with the sample; and (c) observing the optical properties of said pre-formed surface in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material.

25 In a first embodiment of the method of this invention, the optical properties of the pre-formed surface are observed before and after step (b) in order to determine any change in optical properties as a result of the species being bound by the receptive material in
30 the thin film coating on the pre-formed surface. In a second embodiment, the optical properties of the pre-formed surface are monitored during step (b) in order to determine the said change in optical properties.

 According to a second aspect of the present
35 invention, there is provided an article for use in an assay technique as defined above, which article comprises a substrate having a pre-formed surface which is

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1 optically active with respect to radiation at least over
a predetermined band of wavelengths, and at least a
predetermined part of which pre-formed surface is coated
with a thin film of a material capable of binding a
5 predetermined chemical, biochemical or biological
species.

The pre-formed surface is preferably a grating. A
single grating may be employed, or the surface may
comprise two or more gratings disposed mutually at an
10 angle. Where there are two such gratings, they may be
mutually orthogonal. The profile of the or each grating
is advantageously square-wave or sinusoidal. Saw-tooth
profiles are also possible, but are not presently
preferred.

15 The pre-formed surface may alternatively comprise
a regular array of protuberances. With a surface of
this type, the alignment of the peaks of the
protuberances and the troughs between the protuberances
corresponds to the ridges and troughs of a grating-type
20 structure.

The thin film of receptive material may be coated
onto the pre-formed surface so as to be deposited only in
the troughs of the grating or in the troughs between the
protuberances. A monomolecular layer of the receptive
25 material will suffice and will generally be preferred,
whether or not the coating is confined to the troughs.

The surface structure of the pre-formed surface
and in particular the dimensions of the surface relief
pattern will be selected according to the nature of the
30 species which is to be assayed. In general, we have
found three ranges of surface depth (peak-to-trough
measurements) to be advantageous. In the first, the or
each grating or the protuberances has or have a depth in
the range 10 to 50 nanometers. In the second, the depth
35 is in the range 50 to 200 nanometers; and in the third,
the depth is in the range 200 to 2000 nanometers. With
the first of these ranges, the pitch (period) of the or

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- 1 each grating or the periodicity of the protuberances is
advantageously greater than their depth; the structure
thus corresponds, in general, to that of a shallow
grating. With the second and third ranges, the pitch
5 (period) of the or each grating or the periodicity of the
protuberances is advantageously of the same order as
their depth.

In a first group of embodiments, the pre-formed
surface is structured so that it is optically active with
10 respect to radiation whose wavelength is in the range
from 700 to 1500 nanometers. In a second group of
embodiments, the pre-formed surface is structured so that
it is optically active to radiation whose wavelength
falls within the range from 350 to 700 nanometers.

- 15 Conveniently, the substrate which carries the
pre-formed surface is formed of a plastics material.
Plastics materials curable by ultra-violet light are
preferred, and in particular acrylic or polyester
materials can advantageously be used. A presently
20 preferred plastics material is polymethylmethacrylate. A
plastics substrate for use in this invention preferably
has a refractive index in the range 1.25 to 1.6, and more
preferably a refractive index of about 1.4.

An alternative substrate is a glass coated with a
25 synthetic polymeric material.

- The active surface of the substrate (i.e., that
surface which is, or which carries, the pre-formed
surface) can be constituted by a metal or a metal layer.
Thus a plastics substrate, e.g., of polymethylmeth-
30 acrylate, can have adhering thereto a metal layer which
constitutes the pre-formed surface (e.g. a single grating
structure of depth about 250 nanometers and period about
400 nanometers). With such a structure, the
plastics/metal interface may be planar, or it may conform
35 to the surface structure of the metal layer itself. The
metal used to form such layers may be gold, silver,
copper or aluminium. Alternatively, the active surface

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1 of the substrate may be constituted by an inorganic oxide
or a layer thereof. The inorganic oxide is
advantageously an oxide of silver, copper or aluminium.
Such an oxide layer may be produced by causing or
5 allowing oxidation of the surface of a metal substrate or
of a metal layer adhering to a substrate of a different
material. Where there is a layer of metal or of an
inorganic oxide as just described, the layer is
preferably from 5 to 50, more preferably 10 to 30,
10 nanometers thick.

Conveniently, the substrate is lamellar and in
strip-form. This facilitates use of an article in
accordance with the invention in carrying out the assay.

The observation of optical properties in step (c)
15 of the method of this invention can take place in
transmission or in reflection. One zone of the
pre-formed surface on the substrate may be left free of
the coating of receptive material; the method may be
performed by keeping this one zone free from sample, in
20 step (b), or by contacting the whole of the pre-formed
surface, including said one zone, with the sample. This
latter technique has advantages in that any optical
effects caused by components of the sample other than the
species to be assayed will affect the coated and uncoated
25 zones equally, and thus will cancel each other out when a
comparison between the coated and non-coated zones is
made. A two-beam illuminating system can be employed in
step (c) of the method, one of the beams being directed
at the uncoated zone of the pre-formed surface, and the
30 other of the two beams being directed at a part of the
coated zone of the pre-formed surface. Preferably,
monochromatic radiation is used.

When the substrate and the pre-formed surface are
constituted by a plastics material, and observations of
35 the optical properties of the surface are to be carried
out in transmission, it is preferred that the uncoated,
pre-formed surface when viewed in transmission normal to

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1 the plane of the surface should have a transmission not exceeding 1% for the radiation which is to be used.

In order to give optimum results when the technique of this invention is used for quantitative
5 analysis, it may be advantageous to calibrate the coated substrate by first carrying out the assay technique using a sample containing a known proportion of the species which is to be assayed.

The present invention is applicable, for example,
10 to testing a biological liquid, e.g. a blood sample, for specific antigen molecules. In such a case, the receptive material capable of binding the species to be assayed will comprise antibodies for the antigen concerned. Alternatively, it is possible to use an
15 antigen as the receptive material and to assay a sample for antibodies. Where the receptive material comprises antibodies, these are preferably monoclonal antibodies. Antigens and antibodies occur in a wide range of molecular dimensions, and the surface structure of the
20 pre-formed surface will be determined in part by the size of the molecules concerned. As an example, antigens resulting from many parasitic infections are typically in the size range from about 0.5 microns to 10 microns; for these antigens, a grating pitch of greater than 6 microns
25 and preferably greater than 10 microns is desirable. In general, a grating pitch of the order of twice the antigen size will be desirable.

The invention is also applicable to the assaying of other chemical, biochemical or biological species, for
30 example ionic species. The invention may be used, for example, to assay the metal ion content of a sample. The receptive material may be, for example, a chelating chemical or enzyme or a chelating organism which constitutes a specific binding partner for the ligand or
35 ion which is to be assayed. In general, the enzyme or organism will be one or more of: a polypeptide, a steroid, a saccharide or polysaccharide, a proteoglycan,

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1 a nucleotide, a nucleic acid, a protonucleic acid, a
microbial cell or a yeast.

Application of the invention thus lies not only in
the medical field for diagnostics, but also generally in
5 the field of process control.

The thin film of receptive material is preferably
bonded firmly to the pre-formed surface of the substrate.
Thus the receptive material may be bonded by
electrostatic or covalent bonding to said surface.

10 Observations in step (c) of the method of this
invention may use polarised light. In one particular
technique, the pre-formed surface of the substrate is in
the form of a single grating of square-wave or sinusoidal
profile, and the optical properties of the surface are
15 observed, in step (c), by monitoring the angular position
at which there occurs a sharp reduction (dip) in
reflection as the surface is observed or scanned with
polarised radiation of a predetermined wavelength. The
radiation used is preferably light, and the polarisation
20 should be transverse to the grooves of the grating.

A presently preferred article in accordance with
this invention consists of a profiled plastics strip,
desirably fabricated by an embossing or casting
technique, and with a refractive index of the order of
25 1.4 and a transmission not exceeding 1%. The strip
profile may be that of a single grating with square
grooves, dimensioned for zero order suppression over a
range of wavelengths. However, other profiles and
dimensions can be used if desired, enabling diffraction
30 efficiency into particular orders to be enhanced or
suppressed.

An article in accordance with this invention may
have a plurality of zones, each of which is coated with a
different receptive material. In this way, a single
35 article, e.g. in the form of a strip, can be used to
assay a plurality of different species, e.g. antigens in
a blood sample or metal ions in a biochemical fluid or in

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1 an industrial effluent.

In the case of a square profile grating, if the pitch is d , the groove height h and the refractive index n , then zero order diffracted light of wavelength W will
5 be suppressed for $h = W/2(n-1)$, whilst first order diffracted light will emerge at angles given by $\sin \theta = \pm W/d$. For application to blood sampling, given a grating pitch of about 6 microns, and a source wavelength of 550 nm (green), then $h = 0.69$ microns; $\theta = \pm 5.2^\circ$.

10 The principle of the assaying method is that the receptive material, e.g. antibodies, coated on the grating are typically small molecules, e.g. sized around 10 nm, and are too small to produce any size or shape dependent light scattering. However, the antigens
15 attached to the antibodies when a blood sample is smeared on the grating have a size of the same order as the wavelength of incident light, and have an effect analogous to that of filling some of the grating grooves with water (refractive index 1.33). This means that, in
20 the case of a grating dimensioned as above, zero order light is no longer suppressed, whilst very little light is diffracted into the higher orders. Generally, therefore, the transmission of the grating, normally not exceeding 1%, will be directly related to the number of
25 antigens present.

The method thus depends on determination of the change in optical properties, e.g. transmission or reflection characteristics, of the grating. For this reason, given a grating coated with antibodies over its
30 whole area, the smearing of a part of this area with the sample can readily enable the said change to be quantitatively determined. A similar effect is preferably achieved, however, by coating only a part of the grating with antibodies, as the antigens will not be attracted
35 into and trapped in the grooves in the uncoated region. Preferably, in conjunction with the last mentioned partly coated grating, a two beam illuminating system will be

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1 employed. The source may be an incandescent lamp
emitting light incident on the grating through a filter.
The angle of incidence of the monochromatic (or nearly
monochromatic) light on the grating is preferably 0°
5 (i.e. normal to the grating) and, for the grating
exemplified above, zero order diffracted light would be
collected by means of a lens onto a photodetector, while
higher order diffracted light would be obscured using a
stop.

10 One aim of the invention is to provide a low cost
pre-coated grating which can be widely used for
diagnostic purposes, commonly in a general practitioner's
surgery but possibly also in the home. For this
purpose, the antibodies would be firmly bonded to the
15 plastics grating, e.g. by electrostatic bonding which can
ensure virtually permanent coating provided that a
suitable or suitably treated plastics material is
initially chosen to form the grating. As the aim would
usually be to detect a specific antigen, the grating
20 would be coated with a specific antibody, e.g. a
monoclonal antibody which attracts and retains only the
specific antigen in question. Thus, successive testing
of a plurality of selectively coated gratings would
enable quantitative detection of specific antigens as an
25 aid to diagnosis.

The technique of smearing the grating with the
sample also requires consideration. After wiping the
grating with, say, a blood sample, it is important to
remove any excess sample in order to ensure that minimum
30 carrier liquid, minimum haemoglobin and minimum large
cells other than antigen are retained.

As the effect of absorption by red cells
containing haemoglobin can be minimised by suitable
choice of the wavelength of illumination, it is the
35 retention of carrier liquid which is the next likely
source of errors of detection. For minimising such
errors, the grating may be dimensioned for zero order

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1 suppression when there is a continuous liquid film on top
of the grating; this requires a modified grating height
of $h = W/2 (n_1 - n_2)$, where n_1 is the refractive index of
the substrate and n_2 is the refractive index of the
5 liquid. A liquid of high refractive index is desirable,
and one suitable example is glycerol. The smearing
technique (i.e. step (b)) would then include the step of
washing the grating, after wiping it with the sample,
with the liquid in question.

10 A further point to be understood in connection
with the smearing technique is that this will commonly
result in only a small percentage, e.g. less than 2%, of
the overall area of the grating bearing and retaining
attracted antigens. The use of a diffractor grating of
15 high sensitivity relieves the illuminating and detector
system of the extreme requirements which would otherwise
be required quantitatively to detect such a small
presence of antigen, thus making practical the use of
relatively simple and low cost optics which can enable
20 widespread use.

One example of assaying method and apparatus in
accordance with the invention is shown in the
accompanying drawing, in which:

FIGURE 1 shows an optical illuminating and
25 detecting system;

FIGURE 2 shows a detail of one embodiment of an
article incorporating a diffraction grating and forming
part of the system of Figure 1;

FIGURE 3 shows a cross-sectional view (not to
30 scale) of a second embodiment of an article incorporating
a diffraction grating; and

FIGURE 4 illustrates the results obtained with an
article of the type illustrated in Figure 3.

Referring first to Figure 2, a square profile
35 single diffraction grating 10 whose pitch and depth are
both equal to 800 nanometers is coated with a
substantially mono-molecular layer of immobilised

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1 antibodies 12, preferably monoclonal antibodies. After
smearing with a sample, an antigen 14, being a binding
partner to the antibodies, is attracted and trapped in
one groove. At this point of the grating, the zero
5 order diffracted light is transmitted, as indicated at
16, instead of being suppressed.

Figure 1 shows the grating 10 under illumination
by monochromatic light 18. Zero order diffracted light
is collected by a lens 20 onto a photodetector 22, while
10 higher order diffracted light is obscured by a stop 24.
A two-beam illuminating system which, as described above,
is generally preferred, will operate in a precisely
analogous way.

Referring next to Figure 3, an article in
15 accordance with this invention is shown in the condition
after it has been contacted by a sample in step (b) of
the method of the invention. The article comprises a
substrate 10 formed of polymethylmethacrylate which is
about 1 millimeter thick. The active (upper) surface of
20 the substrate includes a layer 11 of aluminium of
thickness 20 nanometers. This is covered by a passive
film 13 of aluminium oxide (thickness one nanometer or
less). A monomolecular layer of antigen molecules 12 is
covalently bonded to the film 13 of aluminium oxide and
25 is thus immobilised. A layer of antibodies 14 is
attached to the antigen layer 12. This layer 14 is also
monomolecular and is about ten nanometers thick.
Isolated antigens 15 have been bound by the antibodies
14. The substrate 10 with the layers 11, 13, 12 and 14
30 constitutes one embodiment of the article of this
invention. The pre-formed surface is in effect defined
by the surface of layer 13; this is in the form of a
single grating of depth 50 nanometers and of pitch
(period) 250 nanometers. The article is observed, in
35 carrying out the method of the invention, with
monochromatic light which is polarised in a plane
perpendicular to the lines of the grating; the angle of

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1 incidence of the illumination is varied and it is found
that there is a sharp reduction (dip) in reflectivity at
an angle whose value depends upon the amount of material
(antibodies 14) overlying the article. The angular
5 position of this dip, and also its angular width, are
strongly dependent upon the amount of antigens attached
to the layer 14 of antibodies and hence these parameters
provide a quantitative measure of the antibodies absorbed
from the sample. Figure 4 plots the reflectivity of the
10 article against the angle of incidence of the
monochromatic, polarised illumination over a small
angular range. As the quantity of antigens captured by
the antibody layer 14 increases, the dip in reflectivity
first of all becomes more pronounced, and then becomes
15 broader and the angular position of the reflectivity
minimum alters, as shown in the three curves plotted.
The reflectivity dip can be considered theoretically as a
plasmon resonance; it is relatively easy to detect a
change in the angle of incidence of about 0.1 degrees or
20 a change in the wavelength of the resonance by about 1
nanometer. Hence it is possible to detect changes
corresponding to an increase in the average thickness of
the antigen layer 15 of around one nanometer. It will
be appreciated that, when antigens are bound by the layer
25 14, the result is not the addition of a further layer of
uniform thickness; nevertheless, we have found that the
occurrence of isolated antigens 15 over the layer 14 of
antibodies behaves approximately as though they were
"smoothed out" into a layer whose average thickness
30 modifies the optical properties of the system as a whole.

1 Claims:

1. An assay technique for qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample, which comprises (a) coating at least a predetermined part of a pre-formed surface on a substrate with a thin film of a material capable of binding the species to be assayed, the pre-formed surface being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) contacting the coated surface with the sample; and (c) observing the optical properties of said pre-formed surface in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material.

2. A method according to claim 1, wherein the optical properties of said pre-formed surface are observed before and after step (b) in order to determine the said change in optical properties.

3. A method according to claim 1, wherein the optical properties of said pre-formed surface are monitored during step (b) in order to determine the said change in optical properties.

4. A method according to claim 1, 2 or 3, wherein said pre-formed surface is a grating.

5. A method according to claim 1, 2 or 3, wherein said pre-formed surface comprises two or more gratings disposed mutually at an angle.

6. A method according to claim 4 or 5, wherein the or each grating is of square-wave profile.

7. A method according to claim 4 or 5, wherein the or each grating is of sinusoidal profile.

8. A method according to claim 4 or 5, wherein the or each grating is of saw-tooth profile.

9. A method according to claim 1, 2 or 3, wherein said pre-formed surface comprises a regular array of

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1 protuberances.

10. A method according to claim 4, 5, 6, 7, 8 or 9, wherein said thin film of material is coated onto the pre-formed surface so as to be deposited only in the
5 troughs of the grating or in the troughs between the protuberances.

11. A method according to any one of claims 4 to 10, wherein the or each grating or the protuberances has or have a depth (peak-to-trough) in the range 10 to 50
10 nanometers.

12. A method according to any one of claims 4 to 10, wherein the or each grating or the protuberances has or have a depth (peak-to-trough) in the range 50 to 200 nanometers.

15 13. A method according to any one of claims 4 to 10, wherein the or each grating or the protuberances has or have a depth (peak-to-trough) in the range 200-2000 nanometers.

14. A method according to claim 12 or 13, wherein
20 the pitch (period) of the or each grating or the periodicity of the protuberances is of the same order as their depth.

15. A method according to claim 11, wherein the pitch (period) of the or each grating or the periodicity
25 of the protuberances is greater than their depth.

16. A method according to any one of claims 1 to 10, wherein said pre-formed surface is structured so that it is optically active with respect to radiation of wavelengths from 700 to 1500 nanometers.

30 17. A method according to any one of claims 1 to 10, wherein said pre-formed surface is structured so that it is optically active to radiation of a wavelength in the range from 350 to 700 nanometers.

18. A method according to any preceding claim,
35 wherein the substrate is formed of a plastics material.

19. A method according to claim 18, wherein said plastics material is a material which is curable by

1 ultra-violet light.

20. A method according to claim 18 or 19, wherein said plastics material is an acrylic or a polyester material.

5 21. A method according to claim 20, wherein said plastics material is polymethylmethacrylate.

22. A method according to any one of claims 1 to 17, wherein the substrate is a glass coated with a synthetic polymeric material.

10 23. A method according to any one of claims 1 to 17, wherein at least the active surface of the substrate is constituted by a metal or a metal layer.

24. A method according to any one of claims 1 to 21, wherein the active surface of the substrate is
15 constituted by an inorganic oxide or a layer thereof.

25. A method according to claim 23, wherein said metal is gold, silver, copper or aluminium.

26. A method according to claim 24, wherein said inorganic oxide is an oxide of silver, copper or
20 aluminium.

27. A method according to any preceding claim, wherein the substrate is in strip-form.

28. A method according to claim 18, 19, 20 or 21, wherein the plastics material has a refractive index in
25 the range 1.25 to 1.6.

29. A method according to claim 28, wherein the refractive index of said plastics material is about 1.4.

30. A method according to any preceding claim, wherein in step (c) the optical properties of said
30 pre-formed surface are observed in transmission.

31. A method according to any one of claims 1 to 29 wherein in step (c) the optical properties of said pre-formed surface are observed in reflection.

32. A method according to any preceding claim,
35 wherein one zone of the pre-formed surface on the substrate is left free of the coating material and is not contacted, in step (b), by the sample.

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1 33. A method according to any one of claims 1 to
31, wherein one zone of the pre-formed surface is left
free of the coating material and the whole of the
pre-formed surface, including said one zone, is
5 contacted, in step (b), by the sample.

 34. A method according to claim 32 or 33, wherein
a two-beam illuminating system is employed in step (c),
one of said beams being directed at said one zone of the
pre-formed surface, and the other of the two beams being
10 directed at a part of said pre-formed surface other than
said one zone.

 35. A method according to any preceding claim,
wherein in step (c) monochromatic radiation is used.

 36. A method according to any preceding claim,
15 wherein the species which is to be detected is an
antigen.

 37. A method according to claim 36, wherein the
material capable of binding said species comprises
antibodies for the antigen which is to be assayed.

20 38. A method according to claim 37, wherein said
antibodies are monoclonal antibodies.

 39. A method according to any one of claims 1 to
36, wherein the species which is to be assayed is an
ionic species.

25 40. A method according to claim 39, wherein said
ionic species is a metal ion.

 41. A method according to claim 39 or 40, wherein
the material capable of binding the species to be assayed
is a chelating enzyme or a chelating organism.

30 42. A method according to claim 41, wherein said
enzyme or organism is one or more of: a polypeptide, a
steroid, a saccharide or polysaccharide, a proteoglycan,
a nucleotide, a nucleic acid, a protonucleic acid, a
microbial cell or a yeast.

35 43. A method according to any preceding claim,
wherein said thin film of material is firmly bonded to
the pre-formed surface of the substrate.

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1 44. A method according to claim 43, wherein said
thin film of material is bonded to the pre-formed surface
of the substrate by electrostatic or covalent bonding.

 45. A method according to any preceding claim,
5 wherein, in step (c), polarised light is used to observe
the optical properties of the pre-formed surface.

 46. A method according to claim 45, wherein the
pre-formed surface of the substrate is in the form of a
single grating of square-wave or sinusoidal profile, and
10 wherein the optical properties of the pre-formed surface
are observed, in step (c), by monitoring the angular
position at which there occurs a sharp reduction (dip) in
reflection as the surface is observed or scanned with
radiation of a predetermined wavelength.

15 47. An article for use in an assay technique as
claimed in claim 1, which comprises a substrate having a
pre-formed surface which is optically active with respect
to radiation at least over a predetermined band of
wavelengths, and at least a predetermined part of which
20 pre-formed surface is coated with a thin film of a
material capable of binding a predetermined chemical or
biochemical or biological species.

 48. An article as claimed in claim 47, wherein
the substrate is a plastics material.

25 49. An article as claimed in claim 48, wherein
said plastics material is a material which is curable by
ultra-violet light.

 50. An article as claimed in claim 48 or 49,
wherein said plastics material is an acrylic or a
30 polyester material.

 51. An article as claimed in claim 50, wherein
said plastics material is polymethylmethacrylate.

 52. An article as claimed in claim 47, wherein
the substrate is a glass coated with a synthetic
35 polymeric material.

 53. An article as claimed in any one of claims 47
to 52, wherein the substrate is lamellar.

1 54. An article as claimed in claim 53, wherein
the substrate is in strip-form.

5 55. An article as claimed in claim 52, 53 or 54,
wherein the pre-formed surface is in the form of a single
grating or of two or more gratings disposed mutually at
an angle.

56. An article as claimed in claim 55, wherein
the or each grating is of square-wave, sinusoidal or
saw-tooth profile.

10 57. An article as claimed in claim 52, 53 or 54,
wherein the pre-formed surface comprises a regular array
of protuberances.

15 58. An article as claimed in any one of claims 47
to 57, wherein said pre-formed surface is constituted by
a metal or a metal layer.

59. An article as claimed in claim 58, wherein
said metal is gold, silver, copper or aluminium.

20 60. An article as claimed in any one of claims 47
to 59, wherein said pre-formed surface is constituted by
an inorganic oxide.

61. An article as claimed in claim 60, wherein
said oxide is an oxide of silver, copper or aluminium.

25 62. An article as claimed in any one of claims 47
to 61, wherein said thin film of material comprises
antibodies.

63. An article as claimed in claim 62, wherein
said antibodies are monoclonal antibodies.

30 64. An article as claimed in any one of claims 47
to 61, wherein said thin film of material comprises a
chelating enzyme or a chelating organism.

65. An article as claimed in claim 48, wherein
the substrate has a refractive index in the range 1.25 to
1.6.

35 66. An article as claimed in claim 65, wherein
the refractive index of the substrate is about 1.4.

67. An article as claimed in claim 48, wherein
the pre-formed surface of the substrate when viewed in

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1 transmission normal to the plane of the pre-formed
surface has a transmission not exceeding 1%.

68. An article as claimed in claim 58 or 59,
wherein the pre-formed surface is constituted by a layer
5 of thickness in the range 5 to 50 nanometers.

69. An article as claimed in any one of claims 47
to 68, wherein the article includes a plurality of zones
each of which is coated with a different receptive
material so that the article is capable of binding a
10 plurality of different species.

70. A method according to claim 1, in which the
pre-formed surface is washed immediately after being
contacted with the sample and before the observations in
step (c).

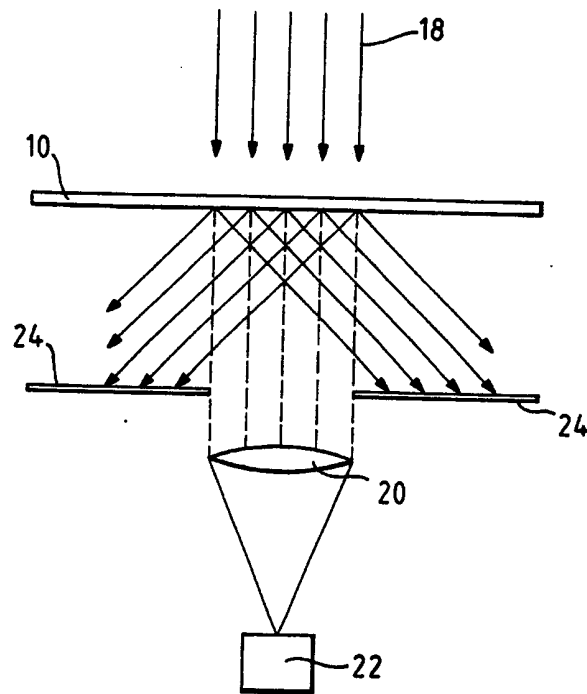
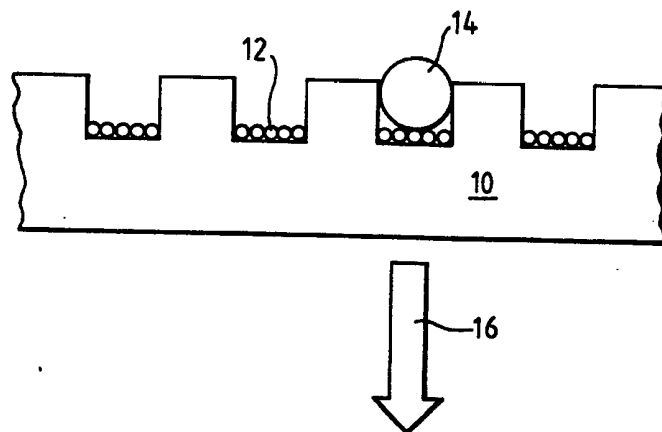
15 71. A method according to claim 1 to 70, in which
the pre-formed surface is covered with a layer of a
liquid of high refractive index between steps (b) and
(c).

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Fig. 1.*Fig. 2.*

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Fig.3.

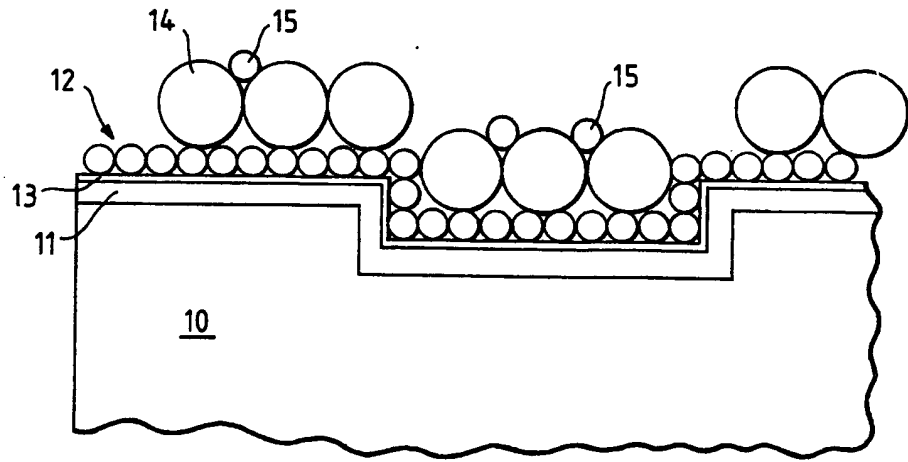


Fig.4.

